REMARKS

FORMAL MATTERS

Applicants have cancelled claims 26-41 and 47 with this Amendment, and have amended claims 23, 48, 50, 51, and 52. Support for the amendment to the claims may be found in the specification on page 6, line 33 through page 7, line 1; page 10, lines 17-23; and page 35, lines 23-34 and in Figure 4A.

WRITTEN DESCRIPTION REJECTION

The Office has maintained the written description rejection of claims 23, 25, and 43-46, and has also rejected claims 48-52. As previously discussed, the Office appears to believe that the invention as claimed is broadly directed to purified HIV-1 variants that differ genetically in the *gag*, *pol*, and *env* coding regions from three known HIV-1 prototypes (IIIB, BRU, and ARV-2) by the specified amounts (at least 3.4% in Gag, 3.1% in Pol, and 13.0% in Env). Further limitations state that AIDS patient antibodies also bind to the Gag, Pol, or Env polypeptides of the HIV-1 variants and the same polypeptides in HIV-1_{MAL} and that the virus can be detected by stringent hybridization to HIV-1_{MAL} cDNA. The Office states that this encompasses a large genus of genotypically/phenotypically unrelated HIV strains.

As previously, the Office emphasizes that it believes that the specification only describes the molecular cloning and characterization of a single novel HIV-1 isolate, LAV-1_{MAL}. The Office believes that the skilled artisan would not have reasonably concluded that the inventors were in possession of any other HIV-1

variant. The Office states that the specification does not provide information on the isolation, characterization, and nucleotide sequence for any other HIV-1 variants.

In the last response, Applicants added hybridization language to the claims in an effort to further describe the genus of variants. Applicants also added new claims 48-52, which recite additional structural limitations on the claimed virus. First, these claims specify that the virus is LAV_{MAL}. Second, claim 48 recites that the virus has the same restriction map as Figure 1, while claims 49 and 50 recite that the virus comprises a protein encoded by specific cDNA sequences. Applicants previously argued that these claims provide yet further structural features of the virus, which are supported by the specification.

The Office continues to believe, however, that the specification only describes LAV-1_{MAL}, a molecular clone of HIV-1_{MAL}, and the Examiner continues to be very concerned that the first claim limitation defines the viruses by their divergence from a standard, rather than their similarity, allowing for greater variation in sequence possibilities.

Again, Applicants assert that the specification encompasses both LAV_{MAL} and variants of this virus, providing sufficient information on the properties of this genus. In addition to the information previously presented, the specification, page 3, lines 5-8, states:

> The RNA of these variants and the related cDNAs derived from said RNAs are hybridizable to corresponding parts of the cDNA of LAV_{MAL}.

Furthermore, it continues on page 35, lines 23-34, by providing structural guidance to define the genus of LAV_{MAL} variants:

> the invention extends to all variants of genomes and corresponding DNA fragments (ORF) having substantially equivalent properties, all of said genomes belonging to retroviruses which can be considered as equivalents of LAV_{MAL}. It must be understood that the claims which follow are also intended to cover all equivalents of the products . . . whereby an equivalent is a product, e.g., a polypeptide, which may distinguish from a product defined in any of said claims, say through one or several amino acids, while still having substantially the same immunological or immunogenic properties.

(emphasis added).

The Examiner suggested that it might have been useful to include a panel of specific- and non-specific MAL monoclonal antibodies to differentiate between the claimed variants and other viruses. *See* Office Action, page 6. Applicants believe that at the time of the invention, one skilled in the art knew how to enrich specific antibodies in a population of antibodies, by depleting the nonspecific antibodies from the mixture. For instance, in the present application, the skilled artisan could either immunoprecipitate the MAL-non-specific antibodies (i.e., antibodies that recognize $HIV\text{-}1_{MAL}$ and the three standard HIV-1 strains) with a mixture of the three standard HIV-1 strains, or retain them on an immunoaffinity column containing a resin bound with the three standard HIV-1 strains. Such antibodies could easily be prepared and used by the skilled artisan; it was not necessary that these antibodies be monoclonal.

Additionally, Applicants wish to remind the Office that at the time this invention was made only three HIV strains were known—LAV_{BRU}, HTLV-3, and ARV-2. Their level of sequence identity was relatively high as shown in Figures 4A and 4B. The two Zairean strains, LAV_{MAL} and LAV_{ELI}, were the first sequenced that differed more significantly from the three standard strains. The sequencing of the genomes of these two Zairean strains showed that they had the same genetic organization as the three standard strains. Therefore, the genetic organization, as defined in the claims, also provides a significant structural limitation to the claimed invention.

Lastly, Applicants believe that percentage divergence (the inverse of percentage homology) is adequate to define the claimed viruses. This nomenclature was used regularly by the skilled artisan during this time period to define and distinguish the different variants of the HIV virus. For instance, the enclosed article refers to LAV, HTLV-III, and ARV viruses and differentiates them by their nucleotide and amino acid sequence divergence. *Ratner et al.*, HTLV-III, LAV, ARV are variants of the same AIDS virus, Nature 313:636-637 (1985) (enclosed). The skilled artisan, therefore, considered percentage divergence to be an adequate structural definition and sufficiently clear to distinguish one viral strain from another.

While Applicants continue to disagree with the Office regarding the support for the claims, Applicants have now amended the claims to recite a range of sequence divergence, for example, the HIV-1 virus may now differ from LAV_{BRU} by from 9.8 to 12.0% in the entire Gag protein. These numbers were derived from

Figure 4A. This establishes a range for the variance to these sequences, and provides yet further structural information and sequence information on the claimed viruses. Applicants believe that this amendment addresses the Examiner's concerns regarding a potentially unlimited percentage divergence, and the claims now recite a range of percentage homology for Gag, Pol, and Env.

Therefore, Applicants request that the Office withdraw this rejection and allow the pending claims.

PRIOR ART REJECTION

The Examiner has again rejected the claims as anticipated by or obvious over *Meyers* (1990), as the Examiner alleges that the claims are not entitled to the benefit of the earlier filed U.S. and foreign applications. *Myers* was published after the U.S. and foreign priority dates (U.S. Appln. Ser. No. 07/038,330, filed April 13, 1987, and European Appln. 86401380.0, filed June 23, 1986). Applicants submit that the specification fulfills the written description requirement and, thus, the *Myers* article, published in 1990, cannot be prior art.

CONCLUSION

Applicants respectfully request that this Amendment under 37 C.F.R. § 1.116 be entered by the Examiner, placing the claims in condition for allowance. Applicants submit that the proposed amendments of the claims do not raise new issues or necessitate the undertaking of any additional search of the art by the Examiner, since all of the elements and their relationships claimed were either

earlier claimed or inherent in the claims as examined. Therefore, this Amendment should allow for immediate action by the Examiner.

Furthermore, Applicants respectfully point out that the final action by the Examiner presented some new arguments as to the application of the art against Applicants' invention. It is respectfully submitted that the entering of the Amendment would allow the Applicants to reply to the final rejections and place the application in condition for allowance.

Finally, Applicants submit that the entry of the amendment would place the application in better form for appeal, should the Examiner dispute the patentability of the pending claims.

In view of the foregoing remarks, Applicants submit that this claimed invention, as amended, is neither anticipated nor rendered obvious in view of the prior art references cited against this application. Applicants therefore request the entry of this Amendment, the Examiner's reconsideration and reexamination of the application, and the timely allowance of the pending claims.

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

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Molecular turnover and memory

SIR — Francis Crick considers in *News and Views* under the title "Memory and molecular turnover" [1] the problem, often overlooked, that although memory operates over periods of years or decades, most macromolecules (with the exception of DNA) turn over with half lives of hours or weeks. Crick sees the dilemma since memory is prolonged and a consequence of inter-synaptic interaction which is dependent on fixed intrasynaptic macromolecules, such as membrane glycoproteins, either singly or more probably in larger aggregates of some form. To sustain memory two alternative strategies are proposed, either the memory macromolecule is immune from turnover (a less likely possibility for Crick) or the memory macromolecules in a synapse can be replaced one at a time without altering the overall state of the memory macromolecular complex.

My response to Crick's interesting challenge lies in the observation that perinuclear (often sided) membrane disposition is required *before* protein catabolism in normal cells takes place [2] and some special association of proteins with cytoskeletal elements may precede routing to the cellular destructive machinery [3,4].

Nerve cells are exquisitely polarized with the cell body (nucleus, polysomes and Golgi) quite spatially distinct from synapse forming processes such as axons or dendrites. Therefore, memory proteins dispatched from around the nucleus into processes by axoplasmic flow would need to retrace their steps for destruction as described in the protein turnover cycle [5]. It is not at all fanciful to suppose that the intricate arborizations of nerve cell processes in the temporal cortex (and everywhere else) have evolved, at least in part, in order to separate and 'immunize' informational macromolecules spatially from the apparatus of molecular turnover. Simply detaching such macromolecules from the neuroskeletal system would suffice (compare refs 2-4), thereby preventing (or slowing) the return of the macromolecules to the perinuclear destructive machinery. Alternatively, selective reversible detachment-attachment to the neuroskeleton would identify populations of proteins (or individual proteins) which are to be routed for destruction and replacement.

Both of the above alternatives could be mediated by protein modifications such as those described by Crick. Likewise both possibilities could operate together in a single neurone. Either way, neuronal evolution could have capitalized on the spatial separation of proteins (in membranes) in the synapses of cellular processes away from the perinuclear destruction apparatus in cell bodies so that information storage is achieved by permanent or temporary macromolecular stabilization.

It is perhaps salutory in an ageing human population that facultative loss, as occurs in Alzheimer's disease, and motor disorder, as seen in Parkinson's disease, might be triggered by the disruption of the status of cell body-synaptic trafficking in cortical neurones and nigro-striated pathways respectively, leading to intraneuronal degradative (degenerative) processes.

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Human B-cell cytotoxic lymphokine priority

SIR — The recent article on the cloning and expression of human lymphotoxin by scientists from Genentech Inc.[1] described work that first came to my attention when a report appeared in *The Guardian* of 6 June 1984 concerning an announcement by the company that they had developed a new cancer drug which did not have side effects. The initial scientific papers from Genentech[2] stated that the lymphotoxin in question had a relative molecular mass (M_r) of approximately 20,000. I therefore thought that it must be a new cytotoxic lymphokine. Some seven years earlier[3,4], I had published a description of a humoral cytotoxic factor produced by a human lymphoblastoid cell line of B-cell lineage derived from a local patient with leukaemia.

Those papers of mine, which characterized the properties of the factor and how it was distinguished from other forms of cell killing, were probably the first well documented studies showing that some human B-lymphoblasts growing *in vitro* produced a cytotoxic lymphokine. In these early papers we also reported that the humoral factor preferentially kills malignant cells and that it had reduced by approximately 50 per cent the incidence of malignancy (fibrosarcoma) in mice[3,4]. Our studies also showed that the cytotoxic factor was a protein with an M_r of 65,000±1,000[5].

In the recent paper in *Nature* by Genentech, the authors state that their initial published result (1984) on the relative molecular mass was incorrect and that the actual M_r of this human lymphoblastoid cell-derived lymphotoxin is 60,000–70,000. This value is so closely similar to the 65,000 M_r we had previously reported in 1980[5], that it now seems extremely likely that the human B-lymphoblast-produced lymphotoxin — cloned recently by Genentech — is identical with the cytotoxic factor first described in 1977[3,4]. It will be for future studies to establish whether the cytotoxic factor described from Cambridge in 1977 and the lymphotoxin described in 1984 in the United States are in fact identical and to explore the efficacy in the treatment of malignant states.

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HTLV-III, LAV, ARV are variants of same AIDS virus

SIR — Retroviruses have been isolated reproducibly from patients with the acquired immunodeficiency syndrome (AIDS), and have been designated human T-cell lymphotropic virus (HTLV) type III[1], lymphadenopathy-associated virus (LAV)[2], or rather recently AIDS-related virus (ARV)[3] by different groups of investigators. Forty-eight independent HTLV-III isolates were originally reported from our laboratory[1], several additional ones since[4,5], and now we have obtained more than 100 independent isolates (S.Z. Sakhuddin *et al.*, in preparation). The recent publications of the complete nucleotide sequence of two HTLV-III proviruses[6], LAV[7], and ARV[8] allows a detailed comparison (see table). Sequences of HTLV-III clones BH5 and BH8 (representing the 5′ and 3′ portions of provirus(es), respectively), clone LAV1a, and ARV-2 are compared to HTLV-III clone BH10. LAV is closely related to HTLV-III clone BH10 and differs in 1.5% of nucleotides and 2.2% of amino acids, while ARV-2 differs in 6.3% of its base pairs and 9.2% of its amino acids from that of HTLV-III clone BH10. These data show that HTLV-III, LAV, and ARV are variants of the same virus. The greater sequence divergence of ARV from HTLV-III is not likely to be a result of errors in sequence determination. First, sequences obtained independently in different laboratories for the same HTLV-III clones were in agreement[6]. Second, multiple clones of ARV isolated from the same cell line infected with a virus isolate from a single individual differ in sequence from one another by only 2 or 3 base pairs (bp)[8]. Third, we have sequenced another proviral clone of HTLV-III derived from another one (RF) of the original 48 isolates reported[1], which differs from BH10 to a similar degree as does ARV (our unpublished observations with B. Starcich, B. Hahn

AIDS virus sequences

	Total nucleotides	Total amino acids	No. and % (in parentheses) of differences compared with HTLV-III clone BH10 sequence: HTLV-III clones BH5/BH8 Nucleotide differences	HTLV-III clones BH5/BH8 Amino acid differences	HTLV-III clones BH5/BH8 Non-conservative amino acid differences*	LAV Nucleotide differences	LAV Amino acid differences	LAV Non-conservative amino acid differences*	ARV Nucleotide differences	ARV Amino acid differences	ARV Non-conservative amino acid differences*
LTR†	634	—	8† (1.6)	—	—	10 (1.6)	—	—	30 (4.7)	—	—
Leader and tRNA PBS†	152	—	4† (3.6)	—	—	5 (3.3)	—	—	14 (9.2)	—	—
gag	1,536	512	19 (1.2)	7 (1.4)	3 (0.6)	46‡ (3.0)	16‡ (3.1)	2 (0.4)	86‡ (5.6)	32‡ (6.3)	8 (1.6)
pol	3,045	1,015	29 (0.9)	12 (1.1)	3 (0.3)	59‡ (1.9)	21‡ (2.1)	5 (0.5)	134‡ (4.4)	51‡ (5.0)	14 (1.4)
sor	609	203	6 (1.0)	4 (2.0)	1 (0.5)	2 (3.3)	0 (0.0)	0 (0.0)	31 (5.1)	20 (9.8)	8 (3.9)
Between *sor* and *env-lor*	584	—	16 (2.7)	—	—	11 (1.9)	—	—	49 (8.4)	—	—
env-lor:											
Signal peptide	111	37	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.8)	2 (5.4)	0 (0.0)	26§ (23.4)	12§ (32.4)	3 (8.1)
Extracellular portion	1,443	481	27‖ (1.9)	12‖ (2.5)	5 (1.0)	32¶ (2.2)	14¶ (2.9)	6 (1.2)	164# (11.4)	82# (17.0)	41 (8.5)
Transmembrane portion	1,035	345	9 (0.9)	6 (1.7)	2 (0.6)	9 (0.9)	5 (1.4)	2 (0.6)	66 (6.3)	42 (12.2)	19 (5.5)
3′ *orf***	648	216	12 (1.8)	5 (2.3)	1 (0.5)	13 (2.0)	8 (3.7)	3 (1.4)	52†† (8.0)	29†† (13.4)	10 (4.6)
Total	9,213	2,593	122 (1.3)	40 (1.5)	14	144 (1.5)	58 (2.2)	15	582 (6.3)	239 (9.2)	93

* Considered conservative substitution if same charge or both neutral, and both either hydrophilic or hydrophobic amino-acid insertions and deletions not counted.
† Portion of R, U5 and leader sequence deleted from unintegrated HTLV-III clones BH5, BH8 and BH10, and determined from integrated clone HXB2.
‡ Deletion of one copy of 36-bp (12 amino acid) direct repeat sequence.
§ Insertion of 9-bp sequence and deletion of 15-bp sequence.
‖ Deletion of 15-bp (5 amino acid) sequence.
¶ Insertion of 15-bp (5 amino acid) sequence.
# Deletion of 18-bp (6 amino acid) sequence, insertion of 12 bp (4 amino acid) sequence, deletion of 6 bp (2 amino acid) and 15 bp (5 amino acid) sequences.
** Includes full open reading frame defined by BH8 and LAV sequences; BH10 has a termination codon at amino acid position 134.
†† Insertion of 12-bp (4 amino acid) sequence.

and G. Shaw).

The DNA sequence data confirm the findings from restriction enzyme site analysis of the HTLV-III genomes which show a spectrum of diversity from closely related to more distantly related variants (ref. 9 and F.W.-S. *et al.*, unpublished). The closer similarity of the LAV DNA sequence to that of HTLV-III might be because the individuals from whom these isolates were derived acquired the virus at a similar time and place. In fact, many of our earliest HTLV-III isolates were all from specimens obtained in late 1982 or early 1983 from the east coast of the United States[1,10] and LAV, although isolated from a French man with a lymphadenopathy syndrome, had his contact in New York in the same period[2]. In contrast, the individual from whom ARV was derived was from California, and the specimen was apparently obtained in 1984[3]. The other more divergent virus (RF) was obtained from a Haitian patient in 1983.

A comparison of the ARV sequence with that of HTLV-III reveals the greatest nucleotide sequence conservation in the LTR and the *gag, pol,* and short open reading frame (*sor*) genes. Many of the differences between the AIDS virus sequences represent in-frame deletions and insertions (see notes at bottom of table). The non-coding areas are somewhat less conserved. The most divergence, however, is within the open reading frame, designated *env-lor*, which encodes the precursor to the envelope proteins and possibly a second protein analogous to *lor* in HTLV-I, HTLV-II, and bovine leukaemia virus (BLV). Of note is the high level of heterogeneity in the extracellular portion of the envelope which differs in 17.0% of its predicted amino acids (8.5% non-conservative amino acid differences) from that predicted for HTLV-III clone BH10. These data may have significant implications for immune system interactions with AIDS virus infected cells, as well as in the design of reagents for viral detection and treatment.

The sequence of clone H9pv22 also derived from HTLV-III-infected H9 cells shows 0.5% nucleotide and 0.6% amino-acid differences from that of clone BH10 (ref. 11).

The presence of a *trans*-acting and transcriptional enhancer in HTLV-III-infected cells, as was demonstrated in HTLV-I, HTLV-II and BLV-infected cells, suggests that there is a gene for *lor* in the HTLV-III genome that may mediate this activity[12]. We have hypothesized that the 3′ portion of the open reading frame designated *env-lor* may be responsible for this function[6], though others have not made a similar interpretation[7,8,11].

The similarities and differences of the AIDS virus isolates to other retroviruses, including the HTLV-BLV family, are discussed in the recent publications[6-8]. The important general structural features of this virus, most notably juxtaposition of myrgagtin, p17, and largagtin, p24 (see ref. 13 for terminology) and the presence of a *lor* coding sequence, suggest that these viruses are related to other members of the HTLV-BLV family. In addition, we are confident that HTLV-III shares nucleotide sequence homology with the *maedi-visna* lenti-retrovirus[14], although another report shows no homology of LAV to *visna*[15]. We cannot explain this descrepancy. A more comprehensive discussion of factors to be considered in taxonomic assignments for this virus is forthcoming (W.A. Haseltine *el al.*, in preparation).

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